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Letters to the Editor

Clinical Impact of Rapid In Vitro Susceptibility Testing and Bacterial Identification

In a recent article by Doern et al. (1), several very important observations were made. This study demonstrated that rapid in vitro identification and susceptibility testing of clinically relevant bacterial isolates by the medical microbiology laboratories result in significant reductions in mortality and costs, which may have profound consequences for the future of clinical microbiology.

However, we have some remarks and questions. Table 6 showed the frequency with which various procedures were performed on patients in the two groups. As we see the number of significant postindex positive blood cultures, the rapid group had 148 tests and the overnight group had 298 tests. It is not clear what the routine policy for the overnight group was and at what moment the preliminary results were given to the clinic.

The authors also report that the overnight test group had a significantly higher mortality rate than the rapid group. Is it possible that the underlying diseases in the overnight group were more serious?

An indication for this could be that the overnight group had significantly more enteric gram-negative bacilli and *Acinetobacter* spp. and the rapid group had significantly more isolates of staphylococci (see data from Table 3 in reference 1) (Table 1).

Our last remark concerns Table 2, which gives the comparison of groups on the basis of primary infectious disease problems. Twenty-one patients in the rapid group and 15 of the patients in the overnight group had line-related bacteremia, for which a P value of <0.0005 is given. However if we recalculate using the chi-square two-sided test, we find a P value of 0.248, which is not significant. It would thus be helpful if the authors could clarify their statistical method of comparison.

TABLE 1. Comparison of patient groups[a]

Organisms	No. (%) of patients in:		
	RAST[b] group	ONAST[c] group	Total
Staphylococci	123 (18)	106 (16)	229 (34)
Gram-negative bacilli	186 (28)	252 (38)	438 (66)
Total	309 (46)	358 (54)	667 (100)

[a] P is 0.007 (chi-square test, two sided).
[b] RAST, rapid antimicrobial susceptibility group.
[c] OAST, overnight antimicrobial susceptibility group.

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Author's Reply

The comments of Dr. Renders and colleagues regarding our clinical impact study are most appreciated. As noted in Table 6, we observed persistently positive blood cultures in 148 patients in the RAST group versus 298 patients in the OAST group. The mean values per patient were statistically significantly different, with a P value of <0.0005. As we stated in Materials and Methods, in both groups AST results on blood culture isolates were phoned directly to physicians immediately after they became available.

In Table 1 and in our discussion on p. 1759 (1), we rigorously attempted to assess underlying disease in the two groups and found no differences between them. The modified criteria of McCabe et al. (2) which we used are a well-established means of accomplishing this. This approach benefits from patient-by-patient assessment of specific baseline disease processes. We also defined patient acuity using Apache scores and found no difference between the two groups. We chose not to present these data because they are predicated on generalizations and really add nothing to the McCabe analysis. Notwithstanding the relatively greater numbers of isolates of *Acinetobacter* and selected members of the family *Enterobacteriaceae* in the overnight group, the two groups were statistically indistinguishable in terms of underlying disease.

We thank Renders et al. for noting the mistake in Table 2. We employed Fisher's exact test for these comparisons. Using this method, the correct P value for line-related bacteremia is 0.228 (i.e., not significant) rather than <0.005.

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Utility of Rapid Monoclonal Antibody-Based Coagglutination Test for Direct Detection of *Vibrio cholerae* O1 and/or O139 in Stool Samples

A monoclonal antibody-based coagglutination test for the rapid diagnosis of *Vibrio cholerae* O139 was evaluated in Bangladesh for the diagnosis of cholera by using 120 watery diarrheal stool samples. By using bacterial culture as the "gold standard," the coagglutination test was found to be 92% sensitive and 100% specific, with 100% positive and 95% negative predictive values (5). Like the mouse monoclonal antibody-based test for *V. cholerae* O1 rapid diagnosis (2), the O139 coagglutination test needs to be improved further. Moreover, a combined rapid nonculture test for detection of *V. cholerae* O1 and *V. cholerae* O139 is required for use in areas in which the disease is endemic as soon as possible. In areas in which *V. cholerae* O139 is endemic, *V. cholerae* O1 infections have not disappeared and exist with *V. cholerae* O139 infections (3). Technical modifications are desirable to increase the sensitivity from 92% (2) to facilitate diagnosis on stool specimens containing less than 10^7 CFU of viable organisms in watery diarrheal specimens. Furthermore, it would be obligatory to detect nonviable *V. cholerae* in field samples in areas in which the disease is endemic. Even though freezing of 30 specimens at −20°C and thawing a week later did not affect the coagglutination results, it would be essential to determine the effects of adverse environment on the performance of the coagglutination test. In areas in which the disease is endemic, apart from high ambient temperatures, there is marked diurnal variation in temperature and humidity. Moreover, extremes of sunlight and humidity and sandstorms and snowstorms have been shown to affect the potency and antigenicity of liquid and freeze-dried vaccines (1). The performance of the coagglutination test might be erratic in areas where ambient temperatures are less than 10°C and facilities for the maintenance of such temperatures around 20°C are not available.

The specificity of the coagglutination test should also be established against the *V. cholerae* O1 strain CVD 103-Hgr, in which virulence has been attenuated by the deletion of the gene for the cholera toxin A subunit, while the gene encoding Hg^{2+} resistance has been maintained (7). The negligible excretion of the attenuated vaccine strain apparent in field trials with children (4) may not be always true for patients with human immunodeficiency virus-induced immunosuppression. The ever-increasing incidence of pediatric AIDS in endemic foci might well pose problems during the use of the newly formulated, inactivated whole-cell plus recombinant B subunit (WC/rBS) cholera vaccine, which was safe and immunogenic when tested in North American volunteers (6). Even oral B subunit–whole-cell (B-WC) vaccines could interfere with and reduce the 100% specificity of the *V. cholerae* O1 and/or O139 coagglutination test.

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Author's Reply

The main utility of the coagglutination test (COAT) for *V. cholerae* O139 Bengal (2) is in field diagnosis of cholera outbreaks. The test may be useful in alerting public health authorities to the occurrence of cholera and mobilizing resources to promptly treat the victims and contain the outbreak. While it is desirable to increase the sensitivity of the test to 100%, at 92% the test is nevertheless useful. In acute cholera, vibrios are excreted in abundance; hence, a COAT with a 92% sensitivity is more than adequate, and testing of each and every stool is not warranted. The key to successful management of a cholera outbreak is a quick diagnosis by testing the stools of a subsample of patients. Once a cholera outbreak is established, all patients should be promptly rehydrated.

One can subject a test to every imaginable environmental condition, but cholera cases are mostly reported in the tropics. When cholera outbreaks occur in remote parts of the world, relief agencies can transport the reagents in cool boxes to these locations and establish a quick diagnosis. In fact, this was amply demonstrated recently by a relief team sent from our center to the Rwandan refugee camps in Zaire, where cholera cases (due to *V. cholerae* O1) were quickly confirmed by the COAT specific for *V. cholerae* O1. Since in the areas of the Indian subcontinent in which cholera is endemic the disease is caused by both *V. cholerae* O1 and *V. cholerae* O139, it is desirable to have a combined rapid test to diagnose both agents of cholera. In fact, such a test (the colorimetric immunoassay) has now been developed and is currently being evaluated. It holds the promise of achieving greater than 92% sensitivity for detection of cholera vibrios (including dead cells). It must be mentioned that COATs for both O1 and O139 vibrios also have the capability to detect dead cells provided they are present at or above the detection limits.

The somatic antigens of O1 and O139 vibrios are not cross-reactive, and the monoclonal antibodies produced against them are reactive to homologous antigens only. Therefore, the *V. cholerae* O139 monoclonal antibody-based COAT will detect only *V. cholerae* O139, and the *V. cholerae* O1 mono-

clonal antibody-based COAT will detect only *V. cholerae* O1 (1).

The meaning of Dr. Arya's last sentence was not clear. However, the COATs for O1 and O139 vibrios are meant to detect these vibrios, whether they be wild strains or vaccine-derived strains, as long as they are excreted above the detection limits of these tests.

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